Department of Agriculture Federated Malay States

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PINK DISEASE.

BY

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Kuala Lumpur, Federated Malay States

October, 1914.

Department of Agriculture, F.M.S.

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INTRODUCTION.

There has been a considerable development of Pink Disease in Malayan rubber estates since 1912 and at the present time it is the disease which requires the greatest amount of attention in many districts. We have seen several estates in which 10 per cent of the trees were affected and in a few neglected plantations no less than 25 per cent were attacked. At the beginning of 1914 it was decided to make a thorough investigation of this disease especially as regards its effect on rubber trees. The present paper embodies the results of this work. A preliminary account of the disease dealing chiefly with the means of identifying the fungus in the field and with the remedial measures to be adopted in dealing with it, was given by one of the present writers (4) in the Agricultural Bulletin of the Federated Malay States for May, 1914.

DISTRIBUTION AND HOSTS.

The disease is caused by a fungus which was named *Corticium javanicum* in 1901 by Zimmermann (17) who investigated it in Java with special reference to Coffee, but Petch (9) points out that the same fungus had been named *Corticium salmonicolor* by Berkeley and Broome many years before from material obtained in Ceylon, hence the latter name has the right of priority and will be used subsequently in this paper.

In 1897 Ridley (11) reported the presence of a disease of Coffee in Selangor caused by a fungus with pink spore masses which was named *Necator decretus* by Massee (8) and in 1901 Zimmermann (17) reported the same fungus on Coffee in Java and pointed out that it was generally associated with *Corticium javanicum*, not only on Coffee but also on Tea, *Bixa orellana* and *Erythroxylon coca*.

In 1904 Ridley (12) reported the presence of a pink fungus on a rubber tree sent from Sandakan. He said he had seen a similar fungus on Ramie and *Strobilanthes* when grown too close together. In 1905 the same fungus was sent to him (13) from rubber trees in Perak and the organism was identified by Massee in 1906 as *Corticium calceum* (14), a European species which is harmless. This identification has since been shewn to be mistaken, the error being probably due to fading of the material during transit to England. In 1909 Gallagher (6) stated that *Corticium Zimmermanni* was associated with a branch and stem disease of rubber trees in Malaya. This name is a synonym for *Corticium javanicum*. In the meantime Pink Disease had been found on rubber in Ceylon (9) and Southern India (1) and it has since been reported on rubber in Sumatra (10) and in Burmah.* *Corticium salmonicolor* on other hosts has been found in the Cameroons (5), and in the Caucasus (15). In the West Indies a pink fungus on cacao has been referred to *Corticium lilacofuscum* which may possibly be identical with *Corticium salmonicolor*.

In 1912 Rant (10) published an account of an investigation of *Corticium salmonicolor* with special reference to its effect on cinchona, the most important result of which was to establish the identity of the *Corticium* and *Necator decretus*, these being shewn to be two stages of one and the same fungus.

Corticium salmonicolor is an omnivorous fungus and Rant mentions that it has been found on no less than 141 species of plants belonging to 104 genera and many different families. The disease has been found on Gymnosperms (*Thuja sp* and *Cupressus glauca, var. pendula*) as well as on Dicotyledons, but no record has yet been made of it on Monocotyledons. Rant (10) states that he has seen the

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*Mr. A. B. Milne recently informed one of us that he had seen the disease on several rubber estates in Southern Burmah.

fungus growing on the epiphytic fern *Drymoglossum heterophyllum* without apparently causing harm.

In Malaya, *Corticium salmonicolor* has been found recently on Para rubber, Cocoa, *Coffea robusta, Gardenia sp., Hibiscus sp.,* Camphor (*Cinnamomum camphora*), *Cassia sp.,* Horse Mango (*Mangifera foetida*), "Langsat" (*Lansium domesticum*), Lime, Durian (*Durio zibethinus*), Jak (*Artocarpus integrifolia*), "Belimbing" (*Averrhoa sp.*), and Mango (*Mangifera indica*). The number of fruit trees in this list is noteworthy, but the fungus is not often found on them.

In other tropical countries the fungus is of economic importance on other plants besides rubber. In Java, coffee and cinchona are seriously affected by it and in Ceylon the fungus causes one of the most serious diseases of tea.

Corticium salmonicolor is probably native in most of the countries in which it has been recorded. Many of the plants mentioned by Rant (10) as having been affected by it are native to Java and some of the plants on which the disease has been found in Malaya are also indigenous. Anstead (1) states that the fungus is present on jungle trees in the neighbourhood of rubber estates in Southern India. The fungus has probably spread from native hosts to plants that have been introduced such as rubber, coffee, tea, and cinchona. There is one doubtful record of it on a jungle tree in Malaya but one cannot say at present whether it occurs to any appreciable extent in the forests. It is rather by good fortune than by systematic searching that the fungus is likely to be found in the jungle. There is presumptive evidence that it does occur in the jungle in this country as in other tropical countries but it is unlikely that Pink Disease causes any serious damage to forest trees. Unfortunately *Corticium salmonicolor* has shewn a considerable liking for rubber trees and as far as Malaya is concerned, *Hevea brasiliensis* is by far the commonest host for the disease.

The chief centres of distribution in the Federated Malay States are Southern Perak and the northern part of Selangor, the district around Pondok Tanjong, and Negri Sembilan, with smaller areas of infection around Teluk Anson, near Kajang, and in Kuala Selangor. In Southern Perak and around Pondok Tanjong most estates have been affected to some extent. During the latter part of 1913 and the early months of 1914 there was a noticeable exten-

sion of the disease downwards from Southern Perak so that estates about 25 miles to the north of Kuala Lumpur are now affected. Between certain of the chief centres of distribution of the fungus there are large tracts of country in which it has not yet been recorded.

It is difficult to account for this distribution of the disease but we can say that it is most abundant at present in the districts of heaviest rainfall and where large tracts of jungle remain. Perhaps the greater development of the disease during 1913 is to be correlated with the particularly heavy rainfall of that year.

In Java the disease caused by *Corticium salmonicolor* is one of the plant maladies which natives speak of as "djamoer upas" (literally, "poison fungus"). Malays of the Federated Malay States appear to have no special name for this disease, but speak of it variously as "chendawan angin" (wind fungus), "chendawan mira muda" (pink fungus), or "chendawan ulat" (worm or caterpillar fungus), the last name having reference to the sinuous course of the threads of the cobweb-like form of mycelium. Planters in Malaya practically always speak of the affection as Pink Disease; occasionally it is called the Java fungus. Petch (9) states that the fungus is sometimes spoken of as the "writing fungus" in Malaya but we have not heard it so called ourselves.

Field Observations.

Pink Disease attacks rubber trees of various ages though it is only rarely seen on trees less than two years old. An attack often begins in a fork of a tree on account of the accumulation of water there but sometimes the disease affects a branch in the middle and it has been seen occasionally to attack the main stem. The disease develops most rapidly during periods of heavy rain. In dry weather obvious signs of the fungus frequently disappear to appear again when the rains come.

The manifestations of Pink Disease on rubber trees are extremely variable. The disease is so called because the fungus often causes a pink incrustation on the branches or main stem, which is more specially developed on the under or shady side. In this condition the disease is very striking and cannot be mistaken. This incrustation cracks irregularly after a time and the bright pink colour rapidly fades to a dingy white. There are, however, at least three other forms in which the fungus appears on rubber trees;—

a. Pink Disease frequently assumes the form of white or pale pink pustules arranged more or less in lines parallel with the branches. This is the "Hockerchen" form of Rant (10).

b. At other times part of the fungus on the exterior consists of white or pale pink strands of a cobweb-like texture which run irregularly downwards over the surface, the strands being sometimes so delicate as to be overlooked (cf. Fig 1). This is the "Spinnegewebe" form of Rant (10) and is usually the first stage to appear on an affected tree.

c. Finally there is the *Necator* stage which was formerly looked upon as a separate fungus, *Necator decretus,* Mass., but is now known to be a stage in the life-history of Pink Disease. Pustules of *Necator* are seen on the left-hand side of Fig 2. The fungus in this condition consists of orange-red, (not pink) pustules about $\frac{1}{8}$ inch in diameter, each pustule being a mass of spores which serve to propagate the disease. In our experience the *Necator* stage has been confined to the side of the branch which is exposed to the brighter light. The *Necator* stage has always been found by us to be associated with other forms of Pink Disease and so intimate is the connection between it and the other forms that it is difficult to understand the doubt that formerly existed as to the identity of *Necator decretus* and *Corticium salmonicolor*.

It often happens that two or more of these stages are present together, but it is important to remember that Pink Disease is by no means always pink in appearance.

Spores of the fungus germinate on healthy bark especially where there is an accumulation of moisture. The mycelium which develops is entirely superficial at first but after a time it penetrates the bark. When the mycelium reaches the laticiferous tissues, exudation of latex frequently begins and this runs down the bark and becomes blackened as time goes on. The weeping of latex from branches is often an indication of the presence of Pink Disease when from ground level no other sign of the disease can be seen. Where latex exudes from the branches, an examination of the cause of outflow should be made and if *Corticium salmonicolor* is foun l to be present the tree

should be treated in the manner described below. Exudation of latex is not however an infallible indication of the presence of Pink Disease; borers often cause weeping, more especially in the lower parts of the tree, abrasions due to wind may be followed by an outflow, and latex sometimes exudes for reasons which are not yet understood. When Pink Disease is present, latex usually flows in a single stream, whereas with borers latex trickles from each hole made by the insects. Once the mycelium has penetrated the bark it rapidly spreads both upwards and downwards over and through the bark causing it to rot. The mycelium spreads more rapidly over the bark than in it. The fungus sometimes advances into the wood, this happening more frequently in small branches than in large ones. If the fungus spreads in the wood the water supply becomes checked and the foliage of the affected branch turns brown and dies. On some undulating estates planters discover those trees which are affected by Pink Disease by observing from a hill the branches which are affected in this manner. Further information concerning the presence and development of the fungus in the wood will be given below. Occasionally the fungus spreads downwards so vigorously that the whole of the upper part of the tree dies. In such cases as those represented in Figs. 3 and 4 the lower part of the tree is making an effort to recover by putting out new branches. When large branches are attacked by *Corticium salmonicolor* the progress of the fungus in the bark may be checked by a spell of dry weather and in this case an open, canker-like wound (compare Fig 5) is often caused as described by Petch (9). The formation of a callus on the margin of the wound tends to repair the injury and occasionally the disease is entirely thrown off, but the fungus sometimes develops again over the bark which began to close the wound. Where the cankered areas have entirely thrown off the disease, the region around them is frequently blackened on account of oxidation of the rubber exuded when the disease was active.

Investigation of *HEVEA* wood affected by *CORTICIUM SALMONICOLOR*.

Previous to Rant's publication (10) Petch (9) stated that *Corticium salmonicolor* does not enter the wood to any appreciable extent. Rant noticed in cinchona that the fungus invaded the wood and the pith. In dealing with

remedial measures he emphasised this fact, indicating that spraying for Pink Disease will be of little use since the hyphae in the wood remain unharmed by the fungicide.

Branches of *Hevea brasiliensis* attacked by Pink Disease sometimes die in a manner characteristic of those attacked by a fungus which grows vigorously in the wood. Young branches are more often affected in this manner than are old ones. The rapid death of the leaves in such branches is a sure sign that the water supply is checked and this restriction is probably due to the activities of the fungus in the wood. It soon became clear that the fungus entered the wood of such branches, so a detailed investigation of the manner in which it invaded these tissues was made. Wood affected by *Corticium salmonicolor* is only slightly discolored and differs greatly in this respect from wood permeated by *Diplodia cacaoicola.*

During the early part of 1914 a large branch of a rubber tree attacked by Pink Disease was obtained from an estate in Negri Sembilan in which the transition between diseased and healthy wood could be clearly traced. The branch was covered with the pink incrustation with ran out below into the cobweb-like form of the fungus. The cortical layers were dead and could be easily stripped off exposing the wood beneath.

When split longitudinally, the wood was seen to be sound for about two feet from the top of the branch but the part below was dry and obviously diseased except for a length of about 9 inches which marked the transition between diseased and healthy wood. This transition area had a moist, almost transparent appearance and gradually passed below into the dry diseased wood and above into the moist healthy wood.

Diplodia cacaoicola, P. Hennings, is a common wound parasite of *Hevea brasiliensis* and has been found in some branches attacked by Pink Disease. A careful search for *Diplodia cacaoicola* was therefore made previous to investigating the mode of attack of *Corticium salmonicolor*. No external sign of *Diplodia* was observed and microscopical examination failed to shew any of the characteristic dark coloured hyphae of this fungus running through the vessels even in the most badly attacked portions of the wood.

Though *Diplodia cacaoicola* was absent, the wood was permeated with hyaline hyphae. A section of the branch including the transition area and a portion of the dead

wood was taken and cut into numbered blocks throughout its length. Razor sections of the wood at different levels were made, the lower portion of the transition area being found most favourable for examination. Transverse sections through this part shew the hyphae ramifying through the elements of the wood, being especially prominent in the vessels (Fig 10). The wood of *Hevea brasiliensis* is mostly composed of fibrous elements together with a comparatively small number of large vessels and narrow medullary rays.

A study of longitudinal sections, both radial and tangential, shews the nature of the attack upon the wood. A favourable radial section shews the hyphae passing transversely through the wood along the medullary rays. As in many tropical trees the medullary rays in *Hevea brasiliensis* are deep and form paths along which the mycelium passes from the bark into the wood. The fungus obtains its nutriment from the food material stored in the medullary ray cells which become filled with septate hyphae (Fig 12). In radial section the medullary rays appear to be broad bands of infected tissue passing through the wood. At places where the vessels meet the medullary rays the mycelium travelling in the cells of the rays spreads out and enters the vessels (cf Fig 11). All the elements of the wood become permeated with the hyphae which pass readily through the large pits without constriction. The deep medullary rays favour a quick passage transversely while the large pits allow a ready passage for the hyphae amongst the elements of the wood.

The most characteristic feature in the wood of *Hevea brasiliensis* when attacked by Pink Disease is the presence of tyloses in the vessels (cf Fig 19). Every specimen examined shewed these bladder-like ingrowths from the living cells bordering the vessels, plugging up the water courses. Specimens of healthy wood, of wood taken from just below the tapping area, and of wood attacked by *Diplodia cacaoicola* were examined but in no case was there any indication of tyloses. Thus the formation of tyloses in *Hevea brasiliensis* appears to be a response to the attacks of *Corticium salmonicolor*.

The tyloses are of two types, (*a*) those in which the cells retain their thin cellulose walls (Fig 17), (*b*) those in which the cellulose walls become lignified (Fig 18). Both types are found in the same branch in adjoining vessels though the second type is rarely produced. The tyloses

which become lignified lose their protoplasm and appear like a number of small vessels included in a larger one. In the majority of cases only the thin walled type is found. Their contents consist of protoplasm and cell sap; no reserve materials are present.

The response made by living cells to an injury usually results in an abnormal growth of neighbouring cells. This is an attempt on the part of the organism to check or repair the damage done by the injury. In *Hevea brasiliensis* attacked by Pink Disease an abnormal bladder-like ingrowth of the living cells bordering the longitudinal path of the fungus, *i.e.* the vessels, takes place.

The formation of tyloses as a definite traumatic response is presumably an attempt on the part of the plant to check the passage of the fungus through the tissues. In this case it is obviously unsuccessful as the hyphae pass easily through the tyloses (Fig 17). Failing this function, however, they are of no further use; in fact they hasten the death of the branch by preventing the ascent of water.

Thus an investigation of the diseased branches explains the symptoms often observed when branches of *Hevea brasiliensis* are affected by Pink Disease. The fungus attacks the wood, vigorously pursuing its course up and down the stem through the vessels. Other vessels are blocked up with tyloses for considerable distances while others again are filled with a brown gummy substance. Thus a large proportion of the vessels are rendered useless. This results in a serious diminution in the amount of water ascending to the leaves which droop and ultimately die.

The features figured and described above are so constant in branches of *Hevea brasiliensis* attacked by Pink Disease that it is difficult to understand how previous investigators failed to observe them. Although Rant in his investigation of the fungus on cinchona was the first to bring forward evidence that *Corticium salmonicolor* entered the wood he did not seem to realise the vigorous nature of its attack in this region.

Description of the Fructifications of the Fungus.

a. Basidial stage.

Zimmermann is responsible for placing the fungus in the genus *Corticium* and one gathers from his description and the accounts of subsequent writers that the pink incrustation is the basidial fructification. We found, however, that this stage was sterile in more than 80 per cent of the large number of cases examined at different times

of the year. Great difficulty was experienced in finding basidia at all and it was not until after much searching that we recognised the type of incrustation that produced basidiospores. This form is considerably thicker, has a more homogeneous surface, and when dry, cracks into larger pieces than the sterile incrustation. It is remarkable that neither Zimmermann, nor other writer on this fungus has called attention to the fact that the pink incrustation is so frequently sterile.

One concludes from Zimmermann's description and figures that a typical hymenium is developed but according to our experience the basidia are scattered and are irregularly arranged as represented in Fig. 9. The size of the spores is as given by Zimmermann and the sterigmata are noticeably long. We have not seen the basidia arranged even approximately as they are in Zimmermann's figure. The irregular distribution of basidia reminds one rather of an *Hypochnus* than a *Corticium*. The fungus certainly differs widely from a typical *Corticium*. Rant (10) has apparently made no special study of the basidial stage as he copies Zimmermann's figure of the hymenium and agrees with his description of it.

In North America, Stevens and Hall (16) have described a disease of pomaceous fruit trees caused by *Hypochnus ochroleucus*, Noack, which spreads over branches and twigs by means of mycelial strands and kills the leaves by enveloping them. The basidia are scattered and are irregular in form. *Corticium salmonicolor* seems to be more similar to *Hypochnus ochroleucus* than to other species of *Corticium*.*

The genus *Necator* was founded by Massee (8) in 1897 for the reception of a single species, *Necator decretus*, which was the cause of a stem disease of coffee trees in Malaya. It is now known that this is one of the stages of *Corticium salmonicolor*. The *Necator* stage consists of orange-red masses of spores, the individual spores being irregular in shape (cf Fig. 14) and hyaline when seen under the microscope. Each spore mass is waxy in consistency and it is likely that the spores become separated from one another only in wet weather when they become washed apart.

The mode of formation of these pustules is different from what one would gather it was by examining Zimmer-

*Bernard has described in a paper entitled "Sur quelques maladies de *Thea assamica*, de *Kickxia elastica*, et de *Hevea brasiliensis*" (Bulletin de Department de l'Agriculture aux Indes Neerlandaises, No 6) a disease of tea in Java caused by a fungus which he named *Hypochnus Theae*. Though there appear to be certain differences between this fungus and *Corticium salmonicolor* the resemblances in the arrangement of the basidia and the character of the sterigmata and spores are very striking.

mann's figures which have been also reproduced in Rant's paper (10). Zimmermann's figures indicate an origin somewhat similar to that of a pycnidium, but we find that in the formation of a *Necator* pustule (Figs 6-8) the mycelium aggregates beneath the outermost layer of cells of the branch forming a kind of stroma, which by growth ruptures the tissues of the host. The whole of this stromatic mass becomes converted into spores by the separation of the cells one from the other. The irregularity in size and shape of the spores (Fig 14) is due to this peculiar method of spore formation. The dimensions of the spores are 14-20 microns x 8-10 microns. In other species of *Corticium* and *Hypochnus* small sclerotia about the size of pustules of *Necator* are produced and in *Corticium salmonicolor* such sclerotial aggregates may have become modified to produce spore masses by separation of the constituent cells instead of forming resting bodies.

Necator spores germinate readily in distilled water and in nutritive solutions (Figs 15 and 16).

The *Necator* has been found much more frequently than the basidial stage and it is likely that it takes the more active part in the dissemination of the disease.

The other forms of *Corticium salmonicolor* are constantly sterile.

The fungus is probably chiefly spread by wind, though it is possible that it is also disseminated by red ants and other insects which visit rubber trees. It is possible too that not only spores but also small portions of the sterile incrustation are disseminated by these means. The incrutation retains its vitality for a considerable time and as stated above it cracks into small pieces as it gets older and these, breaking away, may be carried to other trees in one or other of the ways mentioned.

A species of *Nectria* is often associated with cases of Pink Disease of long standing but as far as is known at present it is purely saprophytic and develops only after the bark has been killed by *Corticium*.

Pure Cultures of *CORTICIUM SALMONICOLOR.*

At the beginning of these investigations efforts to obtain pure cultures of the fungus were unsuccessful. We soon recognised that the incrusting form was usually sterile so we gave up attempts to obtain a deposit of basidiospores

with which to start pure cultures. Failing basidiospores, small pieces of the pink incrustation were cut out with a sterile knife and placed on slants of salep agar. The fungus developed quickly but the cultures were usually contaminated. Subcultures started from these were eventually obtained which were probably pure. No further attempt to obtain pure cultures in this way was made as at this stage one of us obtained material in the field which appeared somewhat unusual at the time. This material bearing orange-red pustules was sent to the laboratory and immediately examined, when the pustules were seen to consist of masses of spores, these being the *Necator* form of the fungus which has since been obtained frequently.

Pure cultures were obtained from the *Necator* spores. These were teased out on sterile glass slides; some were placed in damp chambers on salep agar, others directly on test tube slants of the same medium. The spores germinated quickly in the damp chambers (Figs 15 and 16); these were kept under observation for three or four days in order to see whether the cultures remained pure. After this, transfers from the damp chambers to test tube slants were made. As these cultures and those obtained by placing spores on test tube slants direct were identical, little doubt remained as to their purity. The mycelium was pure white and did not grow copiously. After a period of about ten days a faint pink colouration was to be seen in the cultures. The agar cultures were kept for several weeks but no further development took place. Other cultures were then made by transferring small portions of the mycelium to blocks of sterilised *Hevea* wood placed in tubes (a figure and explanation of the form of the tube used is given in Fig 13). About 50 per cent of the attempts to start cultures from small pieces of mycelium were unsuccessful.

The cultures on wood blocks obtained in the first place from the *Necator* spores develop quickly. The mycelium grows profusely and remains pure white for 7 to 10 days. It spreads over the block and into the cotton wool placed at the base of the tube. Growing in the cotton wool the mycelium begins to turn a pale rose pink colour which gradually spreads over the whole culture. Subsequently the mycelium in the cotton wool becomes intensified to a bright rose colour. The mycelium passes from the cotton wool into the reservoir of water where it becomes aggregated.

The cultures started originally from the pink incrustation were now used to inoculate sterilised wood blocks. The cultures so obtained were identical with those derived from the *Necator* spores so that apart from other evidence this alone indicated the connection between the two forms of the fungus.

The cultures on wood blocks were placed under different conditions to ascertain whether these influenced the development of the colouration. Some were placed on a bench in the laboratory in diffuse light, others in direct sunlight, and others in darkness. Rant (10) states that his agar cultures placed in darkness remained white, though after illumination for a short time a pink colouration appeared. In our experiments the cultures grown in darkness behaved like those in the light except that the pink colour did not develop so rapidly.

In older cultures the mycelium often turns a dirty brown colour as though some impurity had entered. However, small portions of this mycelium seen by transmitted light shew the characteristic colouration. The hyphae in the old brown cultures are closely aggregated, corrugated, septate, much vacuolate, with numerous clamp connections. The hyphae in young cultures are septate, vacuolate, but not corrugated, whilst clamp connections are less numerous.

In one culture placed in bright light and in another kept in diffuse light an aggregation of hyphae took place along the upper edge of the blocks to form a bright pink mass. The aggregation continued till a solid mass $\frac{1}{2}$ inch high and $\frac{1}{2}$ inch in diameter was formed, attached to the block by a thinner base. It resembled a number of closely attached *Necator* pustules (Fig 13). Examined microscopically the mass was found to consist of short cells somewhat irregular in size forming a kind of pseudo-stroma.

These *Necator*-like masses are often formed in the cotton wool at the base of the tube and sometimes upon the surface film of mycelium in the reservoir. Their spore-like nature is indicated when small pieces are used to start new cultures upon wood blocks. In every case there is a copious development of mycelium within 24 hours, while as stated above 50 per cent of attempts to start new cultures with small portions of the usual form of mycelium result in failure. Under natural conditions it is probable that the cells forming these pustular masses would become detached from one another in a manner similar to those forming typical *Necator* pustules.

Rant (10) states he did not obtain either fruit form, *i.e. Corticium* or *Necator* spores in his cultures, but calls attention to the formation of "paraplectenchymatische mycelienknauel" which be obtained now and again in his cultures and which had been previously observed by Koorders (7) in cultures of *Necator*. The *Necator*-like masses obtained by us and described above probably correspond with these structures observed by Rant and Koorders. Rant confined his attention chiefly to agar cultures. In our agar cultures we never obtained anything approaching a fruiting form though our efforts in this direction were not long continued after we found wood blocks so favourable for culture work.

Some of the wood blocks on which cultures had been grown were sectioned. It was seen that the fungus had spread to the centre of the block and that the mycelium had penetrated the elements of the wood in the same manner as in wood naturally affected. Tyloses were absent.

Inoculation Experiments.

Rant's inoculation experiments (10) experimentally demonstrated the connection between *Corticium salmonicolor* and *Necator decretus*. Our inoculation experiments to be now described were carried out to test the conditions which favour or hinder the development of the fungus on *Hevea brasiliensis*. Correlated experiments upon other hosts were kept under observation at the same time.

The first series of inoculation experiments was carried out on rubber trees three and a half years of age. The inoculations were made on the 4th February, 1914, with pieces of the pink incrustation obtained from a diseased rubber tree. The inoculations were covered with cotton wool pads which were moistened every morning for the first three weeks as dry weather was experienced during this period. The pads were kept in place by rubber bands.

Little rain fell between the date of inoculation and the 10th of March, but between March 10th and March 16th there were daily showers. The fungus appeared during this wet spell and the results are given in the following tables:—

The tables summarise the results on March 16th. The inoculations indicated thus * were rendered useless on account of the breaking of the rubber bands. These are not counted in the totals.

HEVEA BRASILIENSIS.

TYPE OF INOCULATION.	TREE.	NUMBER OF INOCULATIONS.	RESULTS.					REMARKS.
			Cobweb-like form.	Incrustation	*Necator.*	*Necator*+Cobweb-like form.	*Necator*+Incrustation.	
	(*a*)	4		(+ —)	+		+	One inoculation on lateral branch 6 ft. above ground : 3 others in forks 10 ft. above ground, close to main stem.
	(*b*)	3		(+ —) +				Two inoculations close to main stem in forks. 1 three ft. from main stem on upper side of branch 10 ft. high : This last one successful
Placed in contact	(*c*)	2*						Two inoculations in main stem 6 ft. high.
	(*d*)	3	+ +			+		Tree with good leaf canopy—very shady—*no cotton wool pads used* : inoculations in forks 15 ft. above ground, close to main stem.
	(*e*)	6						Six inoculations at ends of twigs : 3* obviated due to rubber bands breaking : other 3 unsuccessful.

+ = Successful (+ —) doubtful.

HEVEA BRASILIENSIS.

TYPE OF INOCULATION.	TREE.	NUMBER OF INOCULATIONS.	RESULTS.					REMARKS.
			Cobweb-like form.	Incrustation.	*Necator.*	*Necator* + Cobweb-like form.	*Necator* + Incrustation.	
	(*a*)	3		+	(+ —)			One inoculation four ft. from ground (+ —): other two just above first forks—7 ft. from ground.
	(*b*)	4						None successful: inoculations 15 ft. from ground—bark cut but not sufficiently deep to allow exudation of latex.
Placed in wounds	(*c*)	3*						None successful.
	(*d*)	3 + (3)*						Six inoculations: 3 rubber bands broken: other 3 unsuccessful.
	(*e*)	3	+	(+ —) (+ —)				Wounds made down to wood in first forks about 10 feet above ground.
	(*f*)	3						None successful: wounds deeply cut in twigs.

+ = Successful (+ —) doubtful.

PLACED IN CONTACT.

+	(+ —)	—	Total.
2	1	1	4
1	1	1	3
*	*	*	*
3	—	—	3
0	0	3 + (3) *	3
6	2	5	13

Successful. = $\frac{8}{13}$ = 60%.

PLACED IN WOUNDS.

+	(+ —)	—	Total.
1	1	1	3
—	—	4	4
*	*	*	*
—	—	3	3
1	2	—	3
—	—	3	3
2	3	11	16

Successful. = $\frac{5}{16}$ = 30%.

TOTAL = $\frac{13}{29}$ = 45%.

+ = successful.
(+ —) = doubtful.
— = unsuccessful.

The tables shew that 13 out of 29 inoculations could be accounted successful, i.e. approximately 45 per cent. Of this number 8 out of 13, (60 per cent) were obtained upon uninjured parts; 5 out of 16 (30 per cent) upon wounded surfaces.

Thus the fungus attacks uninjured parts of rubber trees more vigorously than wounded ones. The results provide further evidence that *Corticium salmonicolor* acts rather as a vigorous parasite on uninjured parts than as a wound parasite.

Between March 16th and April 14th the weather was dry and no rain fell for long periods. On the latter date only a few of the trees shewed any trace of the fungus and it appeared either that the fungus was making no progress or was hibernating in the bark.

The inoculations were examined from time to time but the fungus made no further progress. This result may be ascribed to the dry period which followed the moist spell ending on March 16th. A similar result is often observed on estates, for branches of rubber trees may recover from an attack of Pink Disease if a long spell of dry weather intervenes.

Similar experiments were carried out in the field at the same time on *Coffea liberica, Cinchona succirubra, Cinchona ledgeriana,* and *Cinnamomum camphora.* The results are appended in tabular form:—

NAME OF PLANT.	TYPE OF INOCULATION.	NO. OF INOCULATIONS.	RESULTS.
Coffea liberica.	placed in contact.	3	——
	placed in wounds	3	1 successful.
Cinchona succirubra.	placed in contact.	3	——
	placed in wounds.	3	——

NAME OF PLANT.	TYPE OF INOCULATION.	NO. OF INOCULATIONS.	RESULTS.
Cinchona ledgeriana.	placed in contact.	3	—
	placed in wounds.	3	—
Cinnamomum camphora	placed in contact.	3	—
	placed in wounds.	3	—

Only one successful inoculation was obtained and that on *Coffea liberica* where the cobweb-like form of mycelium appeared over the wound in which the fungus had been inserted.

The results indicate that these hosts are attacked less vigorously than is *Hevea brasiliensis,* though the results are only strictly comparable in the case of rubber, coffee, and camphor.

The experiments described above are interesting in that they indicate the possibility that small portions of the incrustation may actively disseminate the disease. These easily break away and are blown by the wind. Under favourable conditions the mycelium may develop and start a new infection.

Inoculations were subsequently commenced in the laboratory with pure cultures of the fungus. Rubber seedlings and plants of *Gardenia sp.* and *Cinchona succirubra* were inoculated either by tying wood blocks used in growing pure cultures upon the stem, or by placing pieces of the mixture of mycelium and cotton wool from the base of the tube, in wounds in the stem or in contact with the stem. The inoculations were made on July 10th and the following table represents the position of affairs on the 19th of August:—

PLANT.	TYPE OF INOCULATION.	WOOD BLOCKS.	MYCELIUM FROM BASE OF TUBES.	RESULT.
Gardenia sp	placed in contact.	3	—	—
Cinchona succirubar.	,, ,, ,,	6	3	—
Hevea brasiliensis. (seedlings).	placed in contact.	6	6	—
	placed in wound	—	6	—

The plants were kept under bell jars and were moistened daily. The inoculation on *Gardenia* and *Cinchona* were overgrown with moulds within a week. In several inoculations on the seedlings of *Hevea brasiliensis*, however, the fungus appeared to be growing strongly at first, but on August 19th the plants were still quite healthy. The experiments indicate that Pink Disease is not likely to attack very young plants of Para rubber.

Inoculations with pure cultures were also carried out in the field. The plants of Hevea stood in an overgrown nursery. Fairly large branches on the outside trees were inoculated on July 14th with the mixture of mycelium and cotton wool from the base of the culture tubes. Similar experiments were carried out at the same time with *Coffea liberica* as host. The table summarises the results on August 19th:—

NAME OF PLANT.	TYPE OF INOCULATION.	NO. OF INOCULATIONS.	NO. OF SUCCESSFUL INOCULATIONS.
Hevea brasiliensis.	placed in contact.	12	1 Cob-web like form
	placed in wounds.	12	1 ,, ,, ,,
Coffea liberica.	placed in contact.	4	1 Cob-web like form
	placed in wounds.	4	—

Thus successful inoculations on both rubber and coffee were obtained with pure culture material. Our object was attained when we had succeeded in establishing the disease from pure cultures.

Rant (10) experimentally demonstrated that *Corticium salmonicolor* does not exhibit the phenomenon of specialised parasitism. Thus this fungus on one host is not limited in infective power to that particular kind of host but can attack a wide circle of other plants. It passes readily under favourable conditions from one host to another. Only in very wet weather does the fungus spread rapidly, as was obvious in our field experiments. Relatively few of our inoculations were successful and this we attribute to the difficulty of keeping them moist during the spells of dry weather which intervened during the progress of the of the experiments.

Treatment.

In the first place it must be pointed out that, exceptional cases apart, spraying with fungicides is impracticable and to some extent also useless. Spraying a rubber plantation with trees 30 to 60 feet high is an entirely different proposition from spraying an orchard containing trees only 20 feet or so high. There would have to be something in the nature of a revolution in spraying methods to enable a mature rubber plantation to be sprayed effectively so as to check Pink Disease. Spraying experiments which we have recently carried out with two up-to-date machines provided with extension rods shew that the maximum height for which they can be used under estate conditions is 25 to 30 feet, and this height is only obtained by coolie labour with difficulty. Longer extension rods have been tried but the difficulties of manipulation under estate conditions are so great that they cannot be used with success for spraying large numbers of trees. Hence if a mature rubber plantation were sprayed to check Pink Disease the upper branches of the trees would remain unprotected and these are the parts most liable to the disease. Again, in view of the regularity of the rainfall in the Federated Malay States a single spraying would be useless. To be effective at all in a climate like this, spraying would have to be repeated at frequent intervals, for it must be remembered that spraying with a fungicide is a preventative rather than a cure. Once Pink Disease has entered the bark the internal mycelium cannot be killed by spraying the exterior.

Spraying with Bordeaux Mixture or Lime Sulphur, preferably the former, might be effective in checking the disease in plantations not more than three years of age if there was danger of it breaking out in epidemic form, but fortunately there is yet no indication of this here. In Southern India where there is a prolonged dry season, Anstead (2) reports that painting the forks of young trees with Bordeaux Mixture before the coming of the monsoon reduced the percentage of trees affected from 1.34 to .56, .07 (three applications of the fungicide were given here), and .7 per cent in various cases. In our opinion estates infected to the extent of about one per cent would be preferably dealt with by cutting out or by tarring as described below. Another circumstance, which would only occur exceptionally, and in which spraying a limited number of trees might be undertaken is where the disease is confined to one portion of an estate. In conjunction with treatment of the disease within the infected area in the manner described below, it might be advisable to spray carefully a belt of trees around this area as a precautionary measure.

Bancroft (3) gives an account of some spraying experiments which he carried out with a view to checking Pink Disease but as he does not give the results of this treatment no conclusions can be drawn. Accounts of spraying experiments unaccompanied by results of treatment are hardly worth of record.

When Pink Disease first appears in a rubber plantation it is usually distributed in a sporadic manner, i.e. one tree is affected here and another one there. It is of the utmost importance that the disease should be dealt with vigorously from the outset by cutting off and burning the affected parts. In most plantations where Pink Disease appears for the first time only a few trees are attacked. In such cases diseased branches should be cut off at least two feet below the lowest point where there are obvious signs of the fungus and it is preferable to cut them off flush with the main stem or larger branch. In no case should "hat pegs" be left. As it is difficult to induce coolies to use saws, care should be taken to see that they cut underneath the branch before there is danger of it being severed from above, otherwise an ugly snag may be left. If the main stem is affected below the level of the lowest branches it should be cut out and burnt.

Where a large number of trees are affected on an estate the manager will probably hesitate before he cuts

out the disease in this drastic manner. As an alternative, branches and main stems which appear to have a chance of recovery should be covered with tar for two feet above and below the region over which the fungus is evident. If the disease is dealt with in this way in the early stages, many branches and sometimes whole trees may be saved. Even when the fungus has penetrated the bark to a slight extent the external application of tar appears to check its progress. It has been urged that the diseased bark should be removed before tar is applied or even that tar should first be placed over the affected parts, the rotten bark removed, and then tar subsequently applied again. These are excellent ideals and if expense were no object would be strongly recommended. Experience has shewn however that where tar is applied thoroughly without previous removal of diseased bark good results are obtained as long as the treatment is renewed within a month if necessary. Trees treated with tar for Pink Disease should be examined within a month and if the fungus has spread, tarring should be tried again. If two applications of tar are found useless in checking the disease the affected parts should be cut out and burnt. On several estates where this mode of treatment has been adopted, Pink Disease has been reduced to a minimum. If tar is used to check Pink Disease it is essential that the work should be done under good supervision for if it is done carelessly the money spent on it will be wasted. It is important also that diseased trees should be treated at an early stage. In certain cases, *e.g.* when the leaves of an affected branch have died, it is obviously hopeless to apply tar. The only thing to do in such cases is to cut out and burn the diseased portions. The use of a concentrated Lime-Sulphur mixture has been tried instead of tar but it is difficult to check the use of it by coolies and it is readily washed off by rain, hence it is not a good substitute for tar and is not recommended.

Planters sometimes have difficulty in burning diseased branches on account of persistent rain. If it is impossible to burn the diseased parts directly, they should be drenched with a 10 per cent solution of Sulphate of Copper, removed from the plantation and buried in the ground some distance away from the rubber trees. It must be remembered however that there is nothing so good as fire for the destruction of fungoid pests. In this connection mention may be made of the fact that another pink fungus, (*Oospora gilva*) which is harmless, usually develops on wood in this

country a few days after it has been burnt. This fungus has been several times mistaken for Pink Disease.

Where Pink Disease has appeared in an estate, a pest gang should be formed if not already established and the size of the gang should be such that it can go over the whole estate and treat diseased trees on the above lines once every three or four weeks. Pink Disease develops rapidly and any longer interval is too great to allow of it being dealt with effectively. Where a considerable amount of Pink Disease is present one can scarcely expect to eradicate it completely, so the expense of maintaining a pest gang must be met. The rubber plantation industry is dependant upon the health of the trees, so it would be suicidal policy to grudge money for treating disease. Fortunately, directors, agents, and managers are alive to this fact.

It sometimes happens that native holders in the neighbourhood of European estates do not clear up pests and diseases on their properties as do the managers of the latter. Such neglect in the case of Pink Disease is doubly serious and it is to be hoped that the Agricultural Pests Enactment will compel offenders to do their duty. Pink Disease is now notifiable (F. M. S. Government Gazette No. 3679, p. 1996, Vol. 5, No. 59 of December 19, 1913).

Any plants besides rubber which are found to be affected with Pink Disease in the neighbourhood of estates should be destroyed as the fungus passes readily from one host to another. The manifestations of the disease on other plants are the same as on rubber.

If the measures indicated above are carried out, the disease should be kept under control, but any neglect of it will be dearly purchased. This disease of rubber trees is more common in Malaya at the present time than the die-back caused by *Diplodia* and it is a disease which will have to be watched most carefully.

In conclusion we wish to express our thanks to Mr. F. de la Mare Norris for kindly making the drawing from which Fig. 13 has been reproduced.

SUMMARY.

1. The distribution, hosts, and mode of action of Pink Disease are described and its importance as a disease of plantation rubber is emphasised.

2. The various forms of *Corticium salmonicolor* are described. It is pointed out that the fungus is not a typical *Corticium* and that the pink incrustation is sterile in the large majority of cases.

3. *Corticium salmonicolor* often affects the wood as well as the bark of rubber trees. Its action on the wood is described in detail. The formation of tyloses appears to be a response to the presence of this fungus in the wood.

4. Pure cultures of *Corticium salmonicolor* have been established on salep agar and on *Hevea* wood.

5. Inoculation experiments both with natural material and with the fungus grown in pure culture have been successful.

6. *Treatment.* *a.* Spraying is not recommended except in particular cases. *b.* The disease is best dealt with either by cutting out infected branches or by treating affected parts with tar. Detailed instructions are given as to the manner in which these operations should be carried out by a pest gang.

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Explanation of Figures.

Fig 1. Photograph of the cobweb-like form of mycelium of *Corticium salmonicolor* on a branch of a rubber tree.

Fig 2. Photograph of incrusting form of *Corticium salmonicolor* (to right) and of pustules of *Necator* stage (to left) on a branch of a rubber tree.

Fig 3. Photograph of rubber tree the upper part of which has been killed by *Corticium salmonicolor*.

Fig 4. ditto.

Fig 5. Photograph of rubber tree bearing "cankers" caused by *Corticium salmonicolor*.

Figs 6-8 Sections shewing stages in development of the *Necator* stage:
(6) stroma just beginning to form under the outer layer of cells. × 200
(7) further development of stroma. × 200
(8) fully developed *Necator* pustule. × 500.

Fig 9. Section of pink incrustation with basidia and sterigmata. Section 6 microns thick and stained with Coton Bleu and Orange G. × 500.

Fig 10. Transverse section of wood shewing hyphae in vessels. × 50.

Fig 11. Longitudinal section shewing hyphae passing from medullary rays into vessels. Hyphae pass through pits without constriction. (Drawing partly diagrammatic). × 200.

Fig 12. Section shewing hyphae in medullary ray cells. × 200.

Fig 13. Culture tube: wood block covered with mycelium which has grown into water reservoir covering the water with a film of hyphae on which *Necator*-like masses have formed. Large *Necator*-like masses at top of wood block.

Fig 14. Group of *Necator* spores, teased out, shewing irregular size of spores. × 500.

Fig 15. Germination of *Necator* spores, after 12 hours in damp chamber. × 500.

Fig 16. Germination of *Necator* spores, after 18 hours in damp chamber. × 500.

Fig 17. Transverse section of vessel with thin-walled tyloses, shewing hyphae passing through tylose cells. × 200.

Fig 18. Transverse section shewing vessel with lignified tyloses. × 200.

Fig 19. Longitudinal section through vessels filled with tyloses. × 200.

Fig. 1.

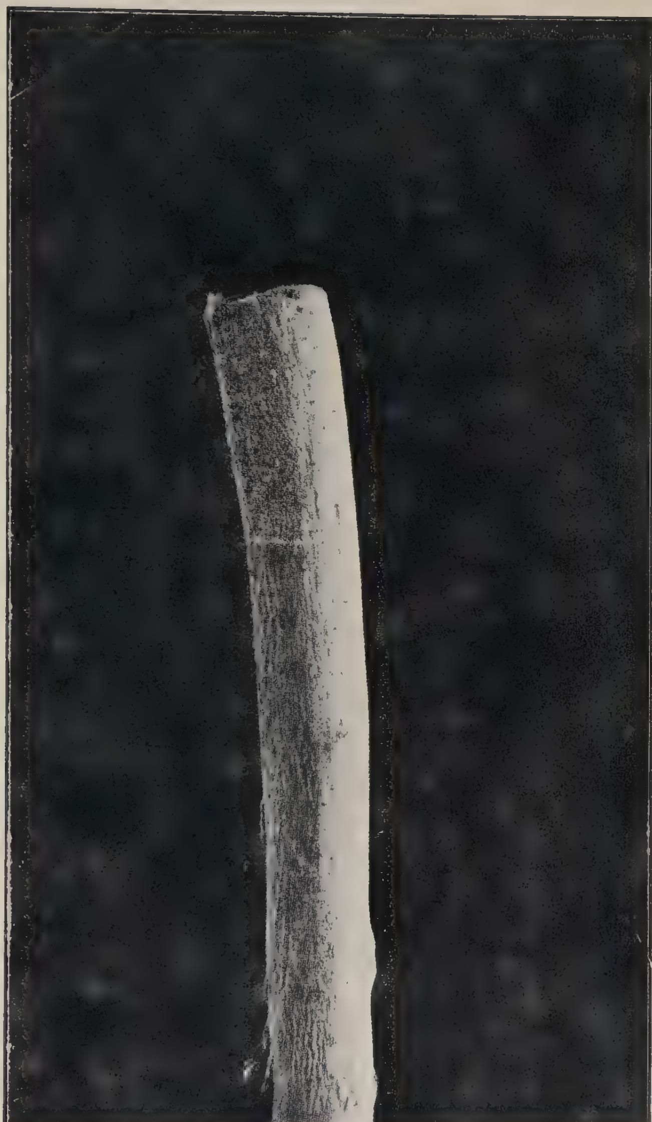

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6

Fig. 7

Fig. 8

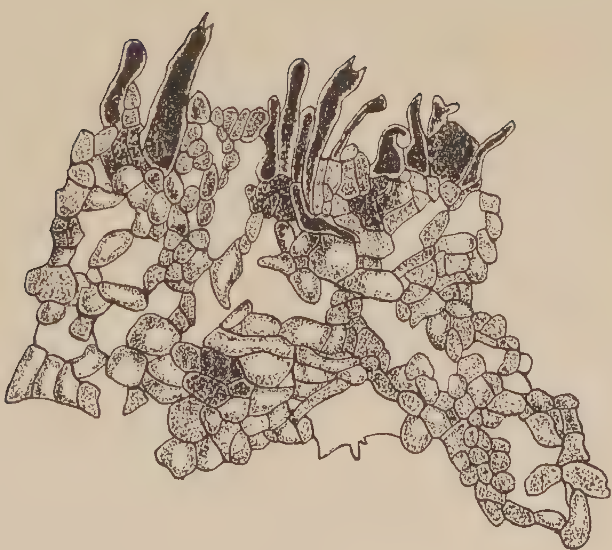

Fig. 9

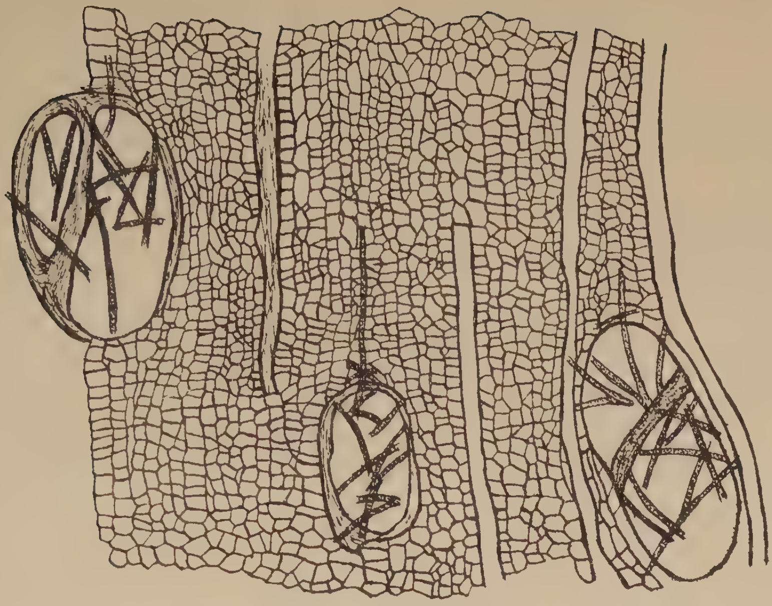

Fig. 10

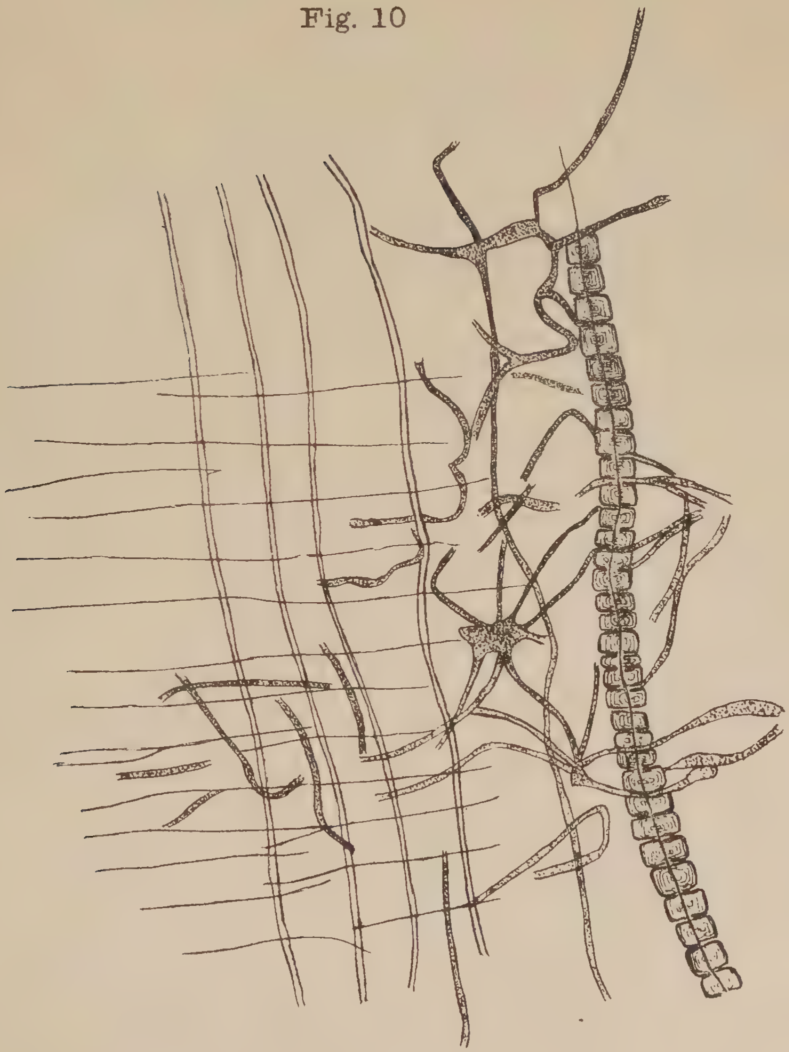

Fig. 11

Fig. 12

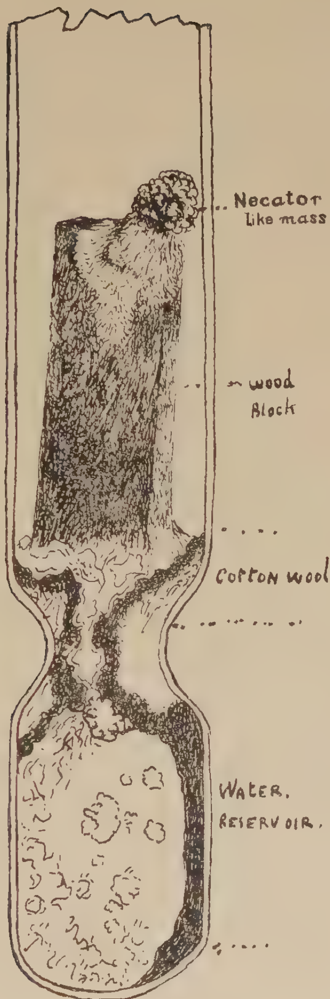

Fig. 13

Fig. 14

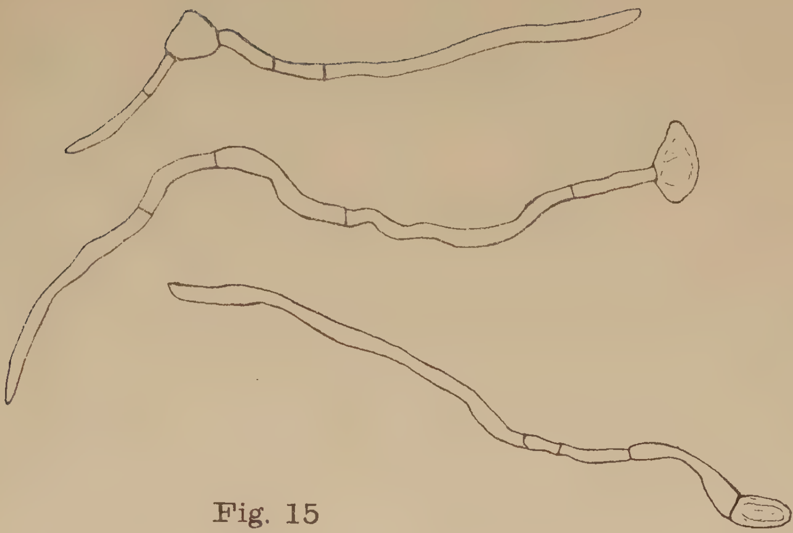

Fig. 15

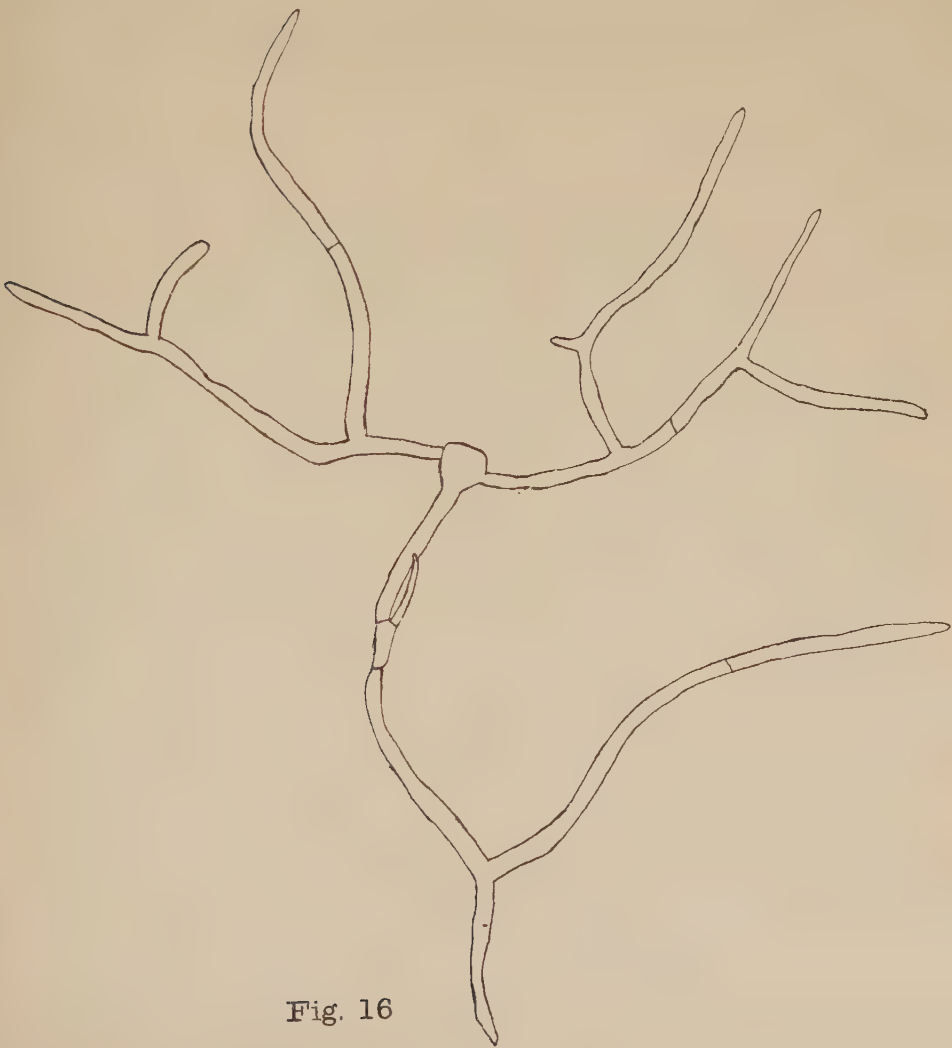

Fig. 16

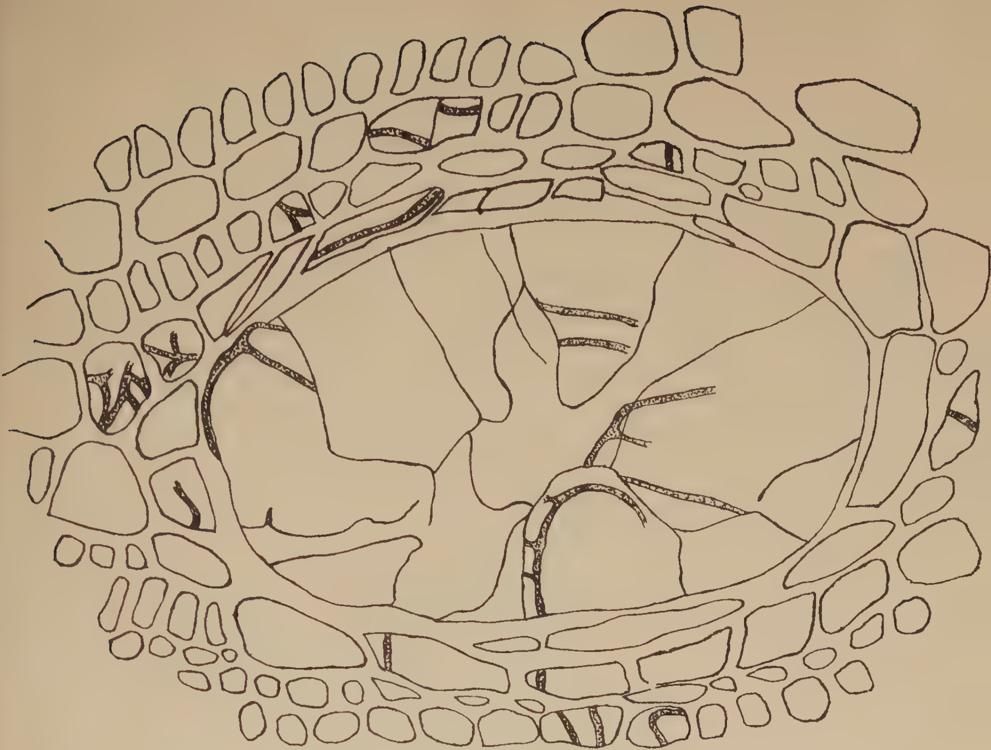

Fig. 17

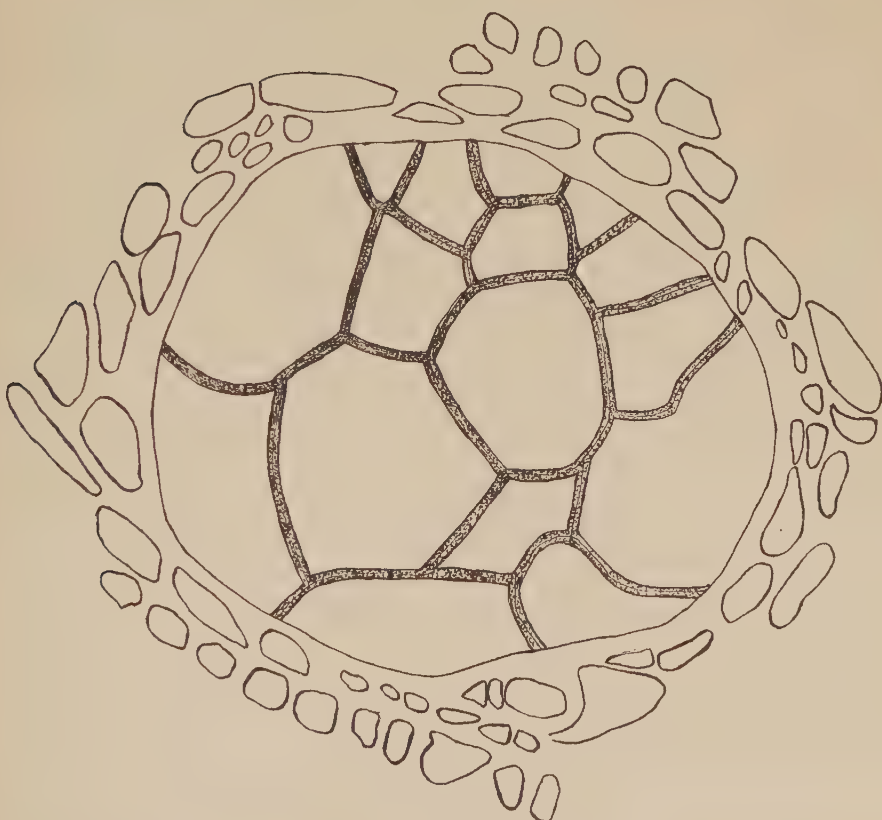

Fig. 18

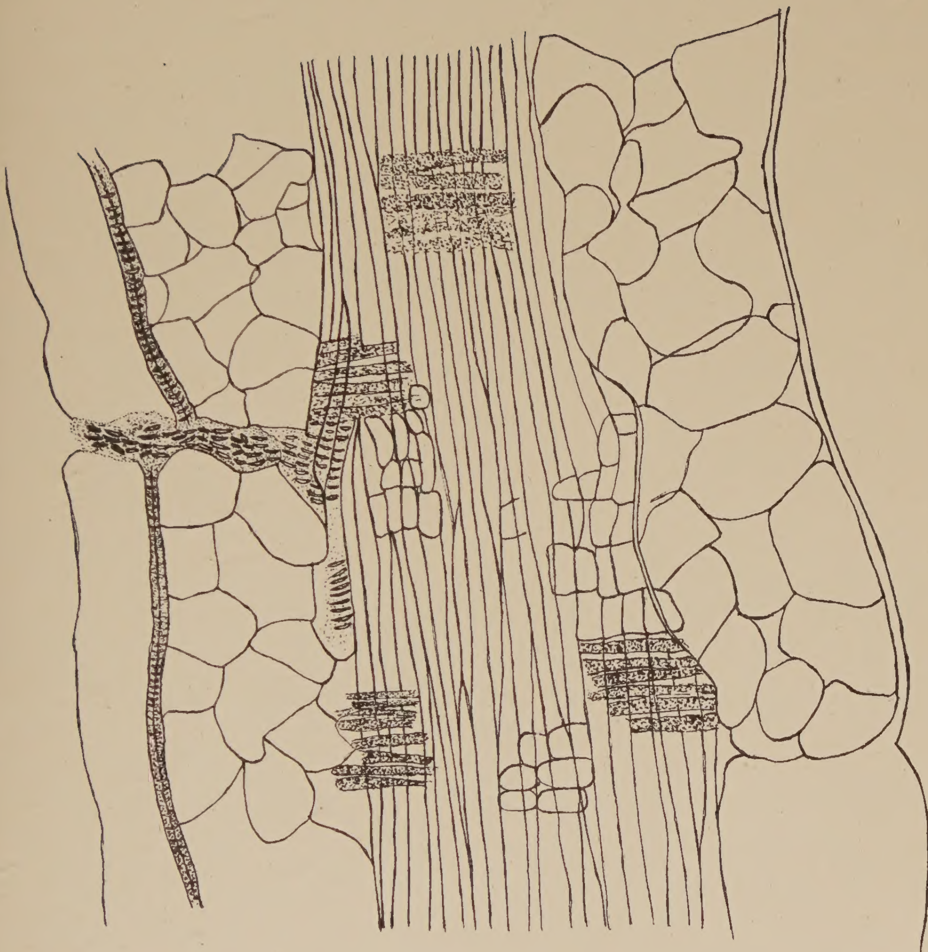

Fig. 19

The following Bulletins have been published by the Department of Agriculture.

1. Notes on *Termes Gestroi* and other species of *Termites* found on Rubber Estates in the Federated Malay States by H. C. Pratt, Government Entomologist. *Out of print*
2. Root Diseases of *Hevea braziliensis*, the Para Rubber Tree: by W. J. Gallagher, Government Mycologist.
3. Observations on *Termes Gestroi* as affecting the Para Rubber Tree, and methods to be employed against its Ravages: by H. C. Pratt, Government Entomologist. *Out of print*
4. A Lepidopterous Pest of Coconuts, *Brachartona catoxantha Hamps*, (Zygaenidae): by H. C. Pratt, Government Entomologist.
5. The Extermination of Rats in Rice-Fields: by W. J. Gallagher, Government Mycologist. *Out of print*
6. A Preliminary note on a Branch and Stem Disease of *Hevea Braziliensis*: by W. J. Gallagher, Government Mycologist. *Out of print*
7. Coffee Robusta: by W. J. Gallagher.
8. The Cultivation and Care of the Para Rubber Tree (in Malay).
9. Die-Back Fungus of Para Rubber and of Cacao: by Keith Bancroft, Assistant Mycologist.
10. A Lecture on the Para Rubber Tree: by W. J. Gallagher. *Out of print*
11. Coconut Cultivation: by L. C. Brown, Inspector of Coconut Plantations.
12. Padi Cultivation in Krian: by H. C. Pratt, Government Entomologist.
13. A Root Disease of Para Rubber, *Fomes Semitostus;* by Keith Bancroft, Mycologist.
14. The Die-back Disease of Para Rubber and a Note on the Leaf Diseases of Para Rubber: by Keith Bancroft, Assistant Mycologist.
15. Camphor: its Cultivation and Preparation in the Federated Malay States, by B. J. Eaton, Agricultural Chemist.
16. The Spotting of Plantation Para Rubber (A Preliminary Account of Investigations on the cause of the Spotting): by Keith Bancroft, Mycologist.
17. The Preparation of Plantation Para Rubber, by B. J. Eaton, Agricultural Chemist.
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19. The Spotting of Prepared Plantation Rubber, by A. Sharples, Assistant Mycologist.
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21. Pink Disease, by F. T. Brooks, Mycologist, and A. Sharples, Assistant Mycologist.